

BIOLOGY OF THE MASSIVE SYMBIOTIC SPONGE *CLIONA NIGRICANS* (PORIFERA: DEMOSPONGIAE) IN THE LIGURIAN SEA

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Cliona nigricans is a boring Atlanto-Mediterranean sponge, which on the Gallinara Island cliffs (Ligurian Sea, Italy), exhibits different growth forms: endolithic specimens bore the coralligenous cliff whereas massive specimens grow on the detritic bottom. In the latter habitat, large massive specimens of *C. nigricans* live partially burrowed in sediment. The sponges incorporate large amounts of sediment, selecting the greater size classes (>5mm). Several incorporated carbonatic fragments, particularly mollusc shells, are bored and crossed by canals of the aquiferous system. The distribution of the massive specimens of *C. nigricans* is affected by the distribution of coarser fractions in the sea bottom sediments. On the detritic bottom *C. nigricans* produces a large extension of secondary solid substrata, hosting a rich biocoenosis of sessile and vagile organisms. Differences in the structure of the aquiferous system between boring and massive stages are shown by corrosion casts, particularly in regard to the shape of exhalant canals. Boring forms possess cylindrical canals while those in massive specimens are moniliform. The density of the symbiotic zooxanthellae, evaluated by chlorophyll analysis of sponge papillae, is related to the seasonal solar radiation and depth. □ *Porifera, Cliona nigricans, growth forms, incorporation, selectivity, boring sponges, zooxanthellae, Ligurian Sea, Italy.*

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There are some species of boring sponges that develop different growth strategies, during their life cycles. After larval fixation, young boring sponges live endolithically with inhalant and exhalant papillae arising from the bored substratum (α form); in the following stage (β form), the papillae progressively form a thin sheet of sponge tissue; when the calcareous substratum is entirely etched away, the sponge grows into a massive form (χ) (Sarà & Vacelet, 1973).

The Gallinara Island (Ligurian Sea, W Mediterranean) hosts a dense population of *Cliona nigricans* which grows from the surface level to the detritic bottom (40-50m depth). The coralligenous cliffs are strongly eroded by *C. nigricans* (α and β forms) producing large tunnels which weaken and fracture the bioherme. At the base of the island cliffs, on the detritic bottom, the χ form of this species grows. The α and β forms are morphologically very different from the χ form, particularly in their exhalant papillae which, in massive forms, have oscular chimneys higher than 10cm. In spite of these morphological differences, electrophoretic analysis has clearly

proven that the two forms belong to the same species (Bavestrello et al., 1996a).

All morphotypes of *C. nigricans* harbour zooxanthellae. This symbiosis is known to be present only in a small number of sponge species (Sarà & Liaci, 1964; Sarà, 1966; Rützler, 1985) whose boring ability has been correlated to the presence of the symbionts (Hill, 1996; Vaclet, 1981).

In this work we consider the relationships of the massive χ forms with the bottom sediment and their influence on the bottom communities in providing secondary solid substrata. Moreover, we compare the symbiont density in these sponges and the anatomy of their aquiferous system to those of boring specimens.

MATERIALS AND METHODS

Cliona nigricans was studied at Gallinara I. (Ligurian Sea), situated about 1.5km from the coast, with underwater cliffs reaching a maximum depth of 37m on the southern side, and a *Posidonia oceanica* bed located between the northern side of the Island and the coast (Fig. 1).



FIG. 1. Density of massive specimens of *Cliona nigricans* (gray areas) around Gallinara I. Key: dark grey >20 specimens/10m²; light grey 5-10 specimens/10m².

The Island consists of greyish quartzitic rocks, together with pelitic layers and cretacic pudding stones (Balduzzi et al., 1994).

The density of massive *C. nigricans* specimens was determined along the eastern, southern and western sides of the islands (Fig. 1) on the detritic bottom at 40-45m depth. Densities were evaluated directly under water by counting the specimens present in a rectangular frame of 10m². The size of some specimens was estimated under water measuring the two main axis of their surface cleaned by sediments. Moreover, the thickness of sediments covering the sponges was measured.

The granulometric features of the bottom detritus (obtained by sieving) were studied on samples collected in areas where sponge density was higher and, for comparison, in areas where massive sponges were absent. In addition, the granulometric characteristics of the bottom sediments were compared to those of the sediments incorporated by the sponges by dissolution of sponge tissues in H₂O₂ (120 vol.). The environmental sedimentation rate was estimated by placing four conical sediment traps in the area with the highest sponge density.

To verify the role of *C. nigricans* in potentially harbouring macrobenthic organisms, specimens were photographed and collected for direct observation in the laboratory. Samples were enclosed in plastic bags and fixed directly underwater in 4% formalin.

Variation in the density of symbiotic zooxanthellae population in *C. nigricans* were determined from fresh tissue samples, taken from peripheral portions of sponges (especially papillae). Sampling took place at different seasons (with five collections made between October 1997 to August 1998), for all morphs and along a bathymetric transect (with five replicas per specimen at 5, 10, 20, 30, 37 and 42m depth). Spectrophotometric analyses of acetic extracts of sponge tissue were conducted according to Gilbert & Allen (1973) to quantify chlorophyll-a concentrations.

Anatomical differences of the aquiferous system in different growth forms were evaluated using corrosion casts (Bavestrello et al., 1995a; Burlando et al., 1990) which were studied under stereomicroscope and, ultrastructurally, by SEM.

RESULTS

Large, massive specimens of *C. nigricans*, growing on the soft detritic bottom, are cushion-shaped or lobate, with a characteristic mamillate surface (Fig. 2C). Their maximal surface ranges from about 200-1000cm² and they are buried in the sediments up to 3-5cm deep. Their inhalant papillae are similar in size and shape to those of endolithic forms (Fig. 2A,B), whereas very high oscular chimneys (up to 10cm high) constitute the exhalant structures. In many specimens the inhalant papillae develop on the wall of the oscular chimney (Fig. 2D).

Tissues of these massive forms are very rich in incorporated bottom sediments which constitute 95% of the sponge dry weight. The mechanism of incorporation appears to be non-selective regarding the origin of foreign bodies: i.e. quartzitic or pelitic particles, rhodoliths, and organogenous detritus are collectively ingested (Fig. 3A-C). Nevertheless, the comparison between the granulometries of bottom sediment and those of sediments incorporated by the sponges, clearly indicates that *C. nigricans* actively selects the coarse fractions larger than 5mm diameter (Fig. 3D).

Corrosion casts demonstrate that calcareous fragments incorporated in the bodies of massive sponges are bored and often crossed by the canals

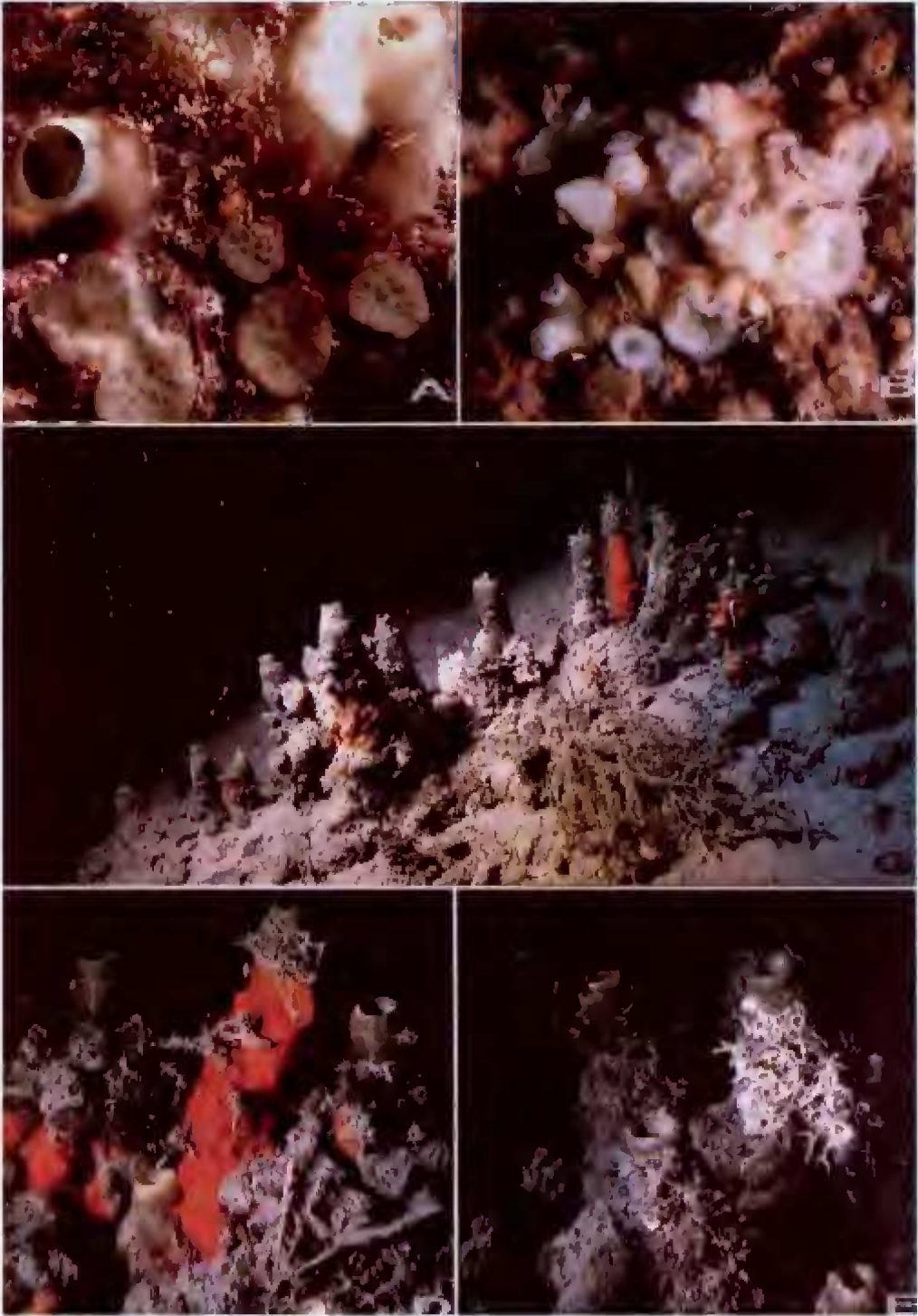


FIG. 2. *Cliona nigricans* specimens. A, Boring α stage. B, Boring β stage. C, Massive χ stage specimens on the detritic bottom characterised by high oscular chimneys and mamillate surface. D, Epibiotic bryozoan *Schizobrachiella* sp.). E, Epibiotic serpulid *Filograna* sp. (E) growing on massive specimens.

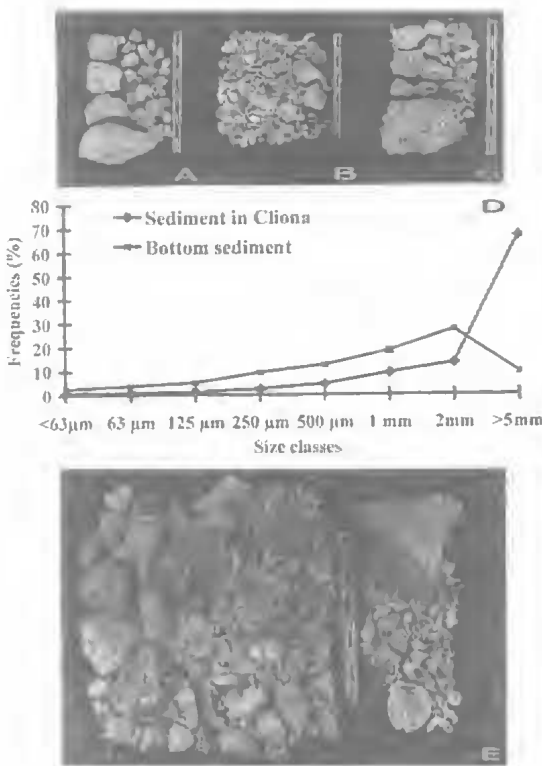


FIG. 3. Incorporation of foreign material by massive specimens of *Cliona nigricans*. A-C, Foreign material incorporated by different specimens. D, Size frequency distribution of material incorporated by sponges compared to that occurring in the surrounding sea bed. E, Fraction of the bottom sediment with a size >5mm occurring where sponge density is highest (left), compared to the same fraction in an area without sponges (right). Scale bars in cm.

of the aquiferous system (Fig. 4A). A comparison between free bottom sediments and those entrapped within the sponge reveals erosion traces in the latter (Fig. 4B-C).

Coarse sediments incorporated by massive specimens derive from fragmentation of the overhanging cliffs, while the thin fraction of terrigenous origin, collected by the traps, reveals an average sedimentation rate of about 10kg/m²/year.

The sponge population density is related to the amount of coarse sediment fraction present in the bottom sediments (Fig. 3E). In areas where sponges show a density greater than 20 specimens/10m² the coarse matter represents 10-15% of the bottom sediments. By comparison, sponges are scarcer (5-10 specimens/10m²) in

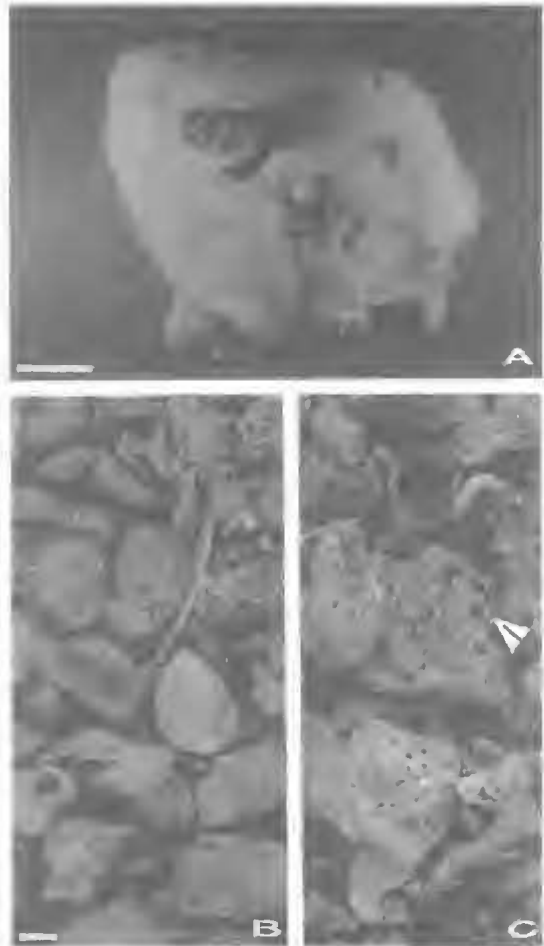


FIG. 4. Boring activity of massive specimens of *C. nigricans*. A, Portion of a corrosion cast of a massive specimen showing a calcareous fragment incorporated by the boring sponge and crossed by an exhalant canal. Scale bar 3mm. B-C, Comparison of the same granulometric fraction of the bottom sediments (B), with those incorporated by the sponges (C). The latter shows evidence of the perforations produced by the sponge (arrow). Scale bar = 8mm.

areas where the coarse fraction is 3-7% of the total, and they are absent where the coarse fraction is less than 3%.

On the soft bottoms of the Gallinara I, massive specimens of *C. nigricans* occupy a large surface on the soft bottom on which they constitute a secondary solid substratum, where a coralligenous-like assemblage lives. This assemblage (Fig. 2C-E) is mainly composed of sessile organisms such as other sponges, hydroids, anthozoans, bryozoans and serpulids that, in turn,

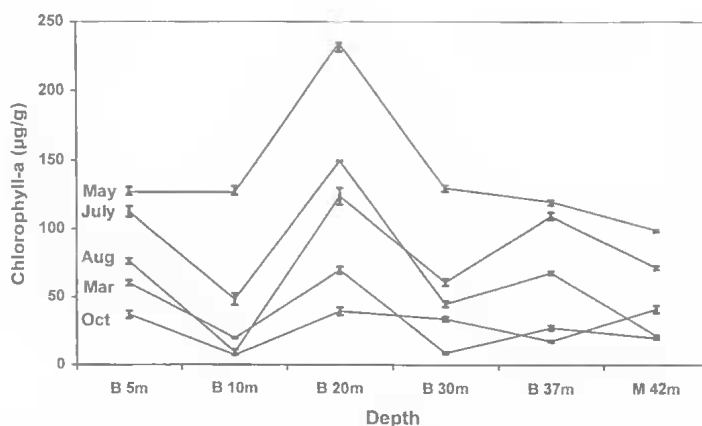


FIG. 5. Average chlorophyll-a concentrations during different periods of the year in specimens living at different depths (N = 5). Key: B = boring specimens; M = massive specimens.

support a vagile fraction represented mainly by nudibranchs, polychaetes, harpacticoids, amphipods and decapods (Table 1).

The concentration of symbiotic zooxanthellae does not vary significantly among massive and boring specimens, but rather exhibits a trend influenced both by season and depth distribution. In October the values are homogeneously low among the different growth forms and at different depths. In March and May these values progressively increase, then subsequently decrease in the following summer months. In all sampling periods a peak in values always occurs at 20m depth (Fig. 5).

Corrosion casts of the aquiferous system reveal differences between the massive and boring specimens in the shape of their exhalant canals. These canals are cylindrical in endolithic specimens (Fig. 6A) and moniliform in massive ones (Fig. 6B). Moreover, endolithic sponges differ from massive ones in the arrangement of choanocyte chambers, which are clustered inside the boring chambers. In massive forms the choanocyte chambers are homogeneously distributed in the sponge body.

DISCUSSION

It is probable that the initial stages of larval development in *Cliona nigricans*, as in all clionids (Sarà & Vacelet, 1973), are linked to the boring activity on a suitable substratum. In coastal detritic bottoms, however, the carbonate fragments are small, and sponge size exceeds the bored fragment very precociously. From this stage, sponge growth is linked to the

incorporation of sediment into its tissue. It is also possible that massive sponges living on the soft detritic bottom originate from the boring specimens higher up on the cliffs which, through fragmentation of the substratum, fall down with a portion of the sponge tissue. In this case, the activity of the boring specimens of *C. nigricans* is an agent for asexual reproduction and spatial dispersal.

Our data indicate that massive specimens of *C. nigricans* select larger fractions (>5mm) of sediment and that high concentrations of these coarse sediments are necessary for successful sponge development. Cellular mechanisms which control this selection are still poorly known (Teragawa, 1986; Bavestrello et al., 1998). The selective ability of sponges to incorporate foreign matter is currently a subject of debate in the literature, with empirical support only recently available (Bavestrello et al., 1995b, 1996b).

Studies in chlorophyll-a concentrations in *C. nigricans* give some indication of the quantitative changes in the symbiotic community of zooxanthellae in relation to depth and seasonal variation. The zooxanthellae population correlates more to the seasonal cycle rather than to depth. Only in

TABLE 1. List of the main phyla living on massive *Cliona nigricans* as sessile epibionts. Key: + occasional; ++ common; +++ present on almost each specimen.

Phylum	Species	Abundance
Porifera	<i>Oscarella lobularis</i>	+
	<i>Dysidea</i> sp.	+
Cnidaria	<i>Clythia hemisphaerica</i>	+++
	<i>C. linearis</i>	+++
	<i>Paralyonium</i> sp.	+
	<i>Caryophyllia smithi</i>	++
Bryozoa	<i>Smitina cervicornis</i>	+
	<i>S. mammillata</i>	+
	<i>Schizobrachiella</i> sp.	+++
	<i>Hippellozoon mediterraneus</i>	+
	<i>Schizomavella auriculata</i>	+++
	<i>Turbicellepora avicularis</i>	+++
Polychaeta	<i>Filograna</i> sp.	++
	<i>Serpula vermicularis</i>	++
Tunicata	<i>Halocynthia papillosa</i>	+

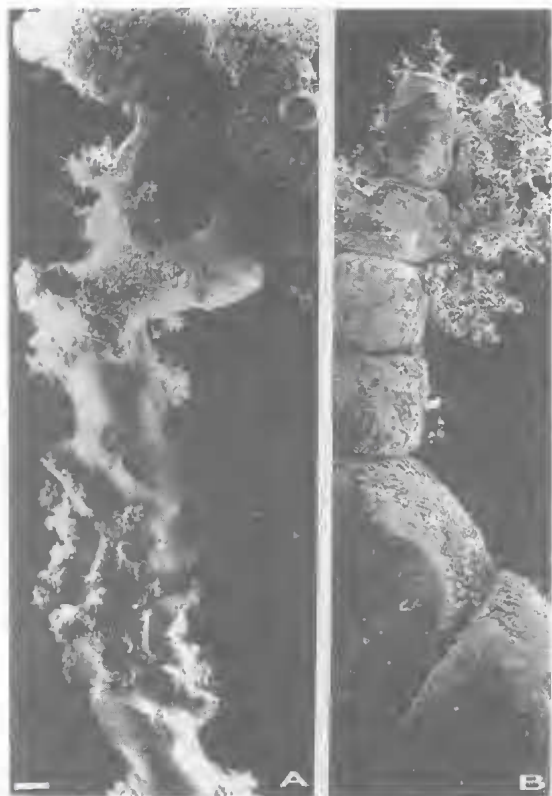


FIG. 6. Corrosion casts made of the aquiferous system of *C. nigricans*. A, Cylindrical shape of boring specimens. Scale bar 250µm. B, Characteristic moniliform structure of the exhalant canals of massive specimens. Scale bar = 100µm.

autumn, the density of zooxanthellae population in massive forms is similar to the density in all boring samples independent from depth. During spring zooxanthellae density increases in all of the sponge morphotypes reaching its maximum in May, in samples collected from 20m depth, and decreasing, subsequently, during the summer. These data indicate significant differences in the behaviour between the zooxanthellae of *C. nigricans* and the cyanobacteria of *Petrosia ficiformis* in the same area (Bavestrello et al., 1992). Cyanobacteria density in *P. ficiformis* is very sensitive to light variations related to depth and, from 10 to 40m, the chlorophyll concentration decreases by about four times. In contrast, the zooxanthellae population in *C. nigricans* remains relatively constant, suggesting a control of the host cells on their reproduction, as in other algae-invertebrate symbioses (e.g. Cook, 1983). Rosell (1993) showed how reproductive process (sexual and

asexual) can modify the density of zooxanthellae in *C. viridis* populations through digestion or expulsion, and how at the end of the sexual process few zooxanthellae were present. Further data are necessary to clarify how reproduction periods affect the population of symbiotic zooxanthellae in *C. nigricans*.

Hill recently (1996) showed how symbiotic zooxanthellae are related to boring activities and growth of a tropical boring sponge (*Anthosigmella varians*). Vacelet (1981) also demonstrated that the most active boring sponges harbour zooxanthellae. Some authors (Hartman, 1958; Sarà & Vacelet, 1973) suggested a decrease in the boring power of endolithic versus massive growth forms, whereas our data indicate that even if fragments, incorporated by massive forms, are widely bored, both endolithic and massive morphotypes have comparable amounts of zooxanthellae.

Differences between the structure of the aquiferous system of boring and massive morphotypes were found through the study of their corrosion casts. The particular beaded shape of the exhalant canals in massive specimens may be determined by a system of contractile elements regularly disposed along the endopinacoderm of the canals.

The two alternative forms of *C. nigricans* (endolithic and massive) are linked to different habitats (coralligenous cliffs and detritic bottoms, respectively), and may be considered in the context of developmental modulation (Smith-Gill, 1983). Morphological variability is common among many sponge species (e.g. Barthel, 1991; Bavestrello et al., 1992), and is generally thought to be linked to variations in the intensity of water movement influencing food supply, and the probability of re-inhalation of filtered waste-water (Fry, 1979). The differences we observed in the behaviour of sponges in relation to their choice of substrata, and of their pumping physiology, between endolithic and massive form of *C. nigricans* stress this variability.

From an ecological perspective *C. nigricans* from Gallinara I. impacts on its environment in two alternative ways: 1) on the cliffs, where boring activity destroys the calcareous substrata, causing fragments to roll down onto the sea floor; and 2) on the detritic bottom, where this same material is gathered up by the massive specimens, which in turn provide a secondary hard

substratum that hosts an unusual biocoenosis, otherwise not present on the soft bottom.

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LITERATURE CITED

- BALDUZZI, A., BIANCHI, C.N., CATTANEO-VIETTI, R., CERRANO, C., COCITO, S., COTTA, S., DEGL'INNOCENTI, F., DIVIACCO, G., MORGIGNI, M., MORRI, C., PANSINI, M., SALVATORI, L., SENES, L., SGORBINI, S. & TUNESI, L. 1994. Primi lineamenti di bionomia bentonica dell'Isola Gallinaria (Mar Ligure). Atti del X Congresso A.I.O.L.: 603-617.
- BARTHEL, D., 1991. Influence of different current regimes on the growth form of *Halichondria panicea* Pallas. Pp. 387-394. In Reitner, J. & Keupp, H. (eds) Fossil and recent sponges (Springer-Verlag: Berlin).
- BAVESTRELLO, G., ARILLO, A., BENATTI, U., CERRANO, C., CATTANEO-VIETTI, R., CORTESOGNO, L., GAGGERO, L., GIOVINE, M., TONETTI, M. & SARÀ, M. 1995b. Quartz dissolution by the sponge *Chondrosia reniformis* (Porifera, Demospongiae). Nature, London 378: 374-376.
- BAVESTRELLO, G., ARILLO, A., CALCINAI, B., CERRANO, C., LANZA, S., SARÀ, M., CATTANEO-VIETTI, R. & GAINO, E. 1998. Siliceous particles incorporation in *Chondrosia reniformis* (Porifera, Demospongiae). Italian Journal of Zoology 65: 343-348.
- BAVESTRELLO, G., BURLANDO, B. & SARÀ, M. 1995a. Corrosion cast reconstruction of the three-dimensional architecture of Demospongiae canal system. Body cavities: function and phylogeny. Pp 93-110. In Lanzavecchia, G. Valvassori, R. & Candia Carnevali, M.D. (eds) Selected Symposia and Monographs. Vol. 8 (Unione Zoologica Italiana, Mucchi: Modena).
- BAVESTRELLO, G., CALCINAI, B., CERRANO, C., PANSINI, M. & SARÀ, M. 1996a. The taxonomic status of some Mediterranean clionids (Porifera: Demospongiae) according to morphological and genetic characters. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie 66 (Suppl.): 185-195.
- BAVESTRELLO, G., CERRANO, C., CATTANEO-VIETTI, R., CALABRIA F., CORTESOGNO L. & SARÀ, M. 1996b. Selective incorporation of foreign material in *Chondrosia reniformis*. Italian Journal of Zoology 63: 215-220.
- BAVESTRELLO, G., PANSINI, M., PRONZATO, R., CATTANEO-VIETTI, R. & SARÀ, M. 1992. Variazioni della concentrazione di clorofilla-a in *Petrosia ficiformis* (Porifera, Demospongiae) con cianobatteri simbiotici. Atti del X congresso A.I.O.L.: 327-331.
- BURLANDO, B., BAVESTRELLO, G. & SARÀ, M. 1990. The aquiferous system of *Spongia officinalis* and *Cliona viridis* (Porifera) based on corrosion cast analysis. Bollettino di Zoologia 57: 233-239.
- COOK, K.B. 1983. Metabolic interaction in algae-invertebrate symbiosis. Pp. 177-210. In Jeon, K.W. (ed.) International Review of Cytology. Supplement 14. (Academic Press: London, New York, San Francisco).
- FRY, W.G. 1979. Taxonomy, the individual and the sponge. In Larwood, G. & Rosen, B.R. (eds) Biology and Systematics of colonial organism (Academic Press: London, New York, San Francisco).
- GILBERT, J.J. & ALLEN, L.H. 1973. Chlorophyll and primary productivity of some green fresh-water sponges. Hydrobiologia 58: 633-658.
- HILL, M.S. 1996. Symbiotic zooxanthellae enhance boring and growth rates of the tropical sponge *Anthosigmella varians* forma *varians*. Marine Biology 125: 649-654.
- HARTMAN, W.D. 1958. Natural history of the marine sponges of Southern New England. Bulletin Peabody Museum of Natural History, Yale University 12: 1-155.
- ROSELL, D. 1993. Effects of reproduction in *Cliona viridis* (Hadromerida) on zooxanthellae. Scientia Marina 57: 405-413.
- RÜTZLER, K. 1985. Association between Caribbean sponges and photosynthetic organisms. Pp. 455-466. In Rützler, K. (ed.) New perspectives in sponge biology. (Smithsonian Institution Press: Washington D.C.).
- SARÀ, M. 1966. Associazioni fra Poriferi e alghe in acque superficiali del litorale marino. Ricerca Scientifica 36: 277-282.
- SARÀ, M. & LIACI, L. 1964. Symbiotic association between zooxanthellae and two marine sponges of the genus *Cliona*. Nature, London 203: 321.
- SARÀ, M., & VACELET, J. 1973. Ecologie des Demosponges. Pp. 462-576. In Brien, J.P. et al. (eds) Traité de Zoologie. III. Spongiaires. Sér. Ed. Grassé, P. (Masson et Cie: Paris).
- SMITH-GILL, S.J. 1983. Developmental plasticity: developmental conversion versus phenotypic modulation. American Zoologist 23: 47-55.
- TERAGAWA, C.K. 1986. Sponge dermal membrane morphology: Histology of cell mediated particle transport during skeletal growth. Journal of Morphology 190: 335-348.
- VACELET, J. 1981. Algal-sponge symbioses in the coral reefs of New Caledonia: a morphological study. Proceedings of the Fourth International Coral Reef Symposium, Manila 2: 713-719.